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# Lyophilized Platelets: Challenges and Opportunities

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Live platelets have many complex functions other than clot formation. These include the modulation of fibrinolysis, inflammation, vascular tone, and cellular growth through substances released from cytoplasmic granules or direct interaction with endothelial cells, leukocytes, and macrophages. Many of these functions are poorly understood and serve as a reminder of the complexity of the cell that researchers seek to mimic.[1]

Despite improvements in the production of 22°C liquid-stored platelets, conventional PCs have several drawbacks. Recipients continue to be at risk for febrile nonhemolytic transfusion reactions; the transmission of bacterial, viral, and protozoan infections; alloimmunization resulting in refractoriness to future platelet transfusions; and graft-versus-host disease. The potential for transfusion-associated immunosuppression has also been a concern. Shortages in platelet supply occur frequently, and the stockpiling of platelets is not feasible because of the short shelf life of conventional platelet concentrates. The result is often wastage owing to the expiration of platelet products, which is inevitable under current transfusion medicine practices. The decline in the donor pool and increase in platelet use over the past decade compounds the problem. These drawbacks have led to efforts to minimize the exposure of recipients to allogeneic blood products and to develop safe and effective platelet products and substitutes with longer shelf lives.[2]

Ideally, modified platelet products and substitutes should function hemostatically as live platelets do without causing pathologic thrombosis or a consumptive coagulopathy. A platelet substitute should not transmit infection, nor should it be immunogenic or cause reticuloendothelial blockade. Novel platelet products and platelet substitutes should have a long duration of action to allow long dosing intervals. Preferably, they should have simple storage requirements (such as room temperature), have a long shelf life, and be easy to administer (off-the-shelf administration or reconstitution in conventional crystalloid solutions and administration without washing).

Although a number of methods are currently under development to improve the storage and safety of liquid-stored platelets, freeze-dried platelets have generated particular interest in the research community. In the 1950s, several investigators reported the effectiveness of rehydrated lyophilized platelets in a small number of thrombocytopenic patients.[3,4] These reports were followed by controlled studies in thrombocytopenic animals that failed to confirm the hemostatic efficacy of reconstituted lyophilized platelets.[5,6] Thereafter, reports of lyophilized platelets suffered a long absence from the literature. More recently, a lyophilized platelet preparation has emerged with more encouraging preclinical results.

A new method for preparation of lyophilized platelets has recently been described.[7] Freeze-dried platelets retain native von Willebrand factor-mediated adhesion and surface thrombin generation functions, whereas coupling of thrombin receptors to integrin inside-out signaling is largely inhibited. This would suggest that freeze-dried platelets may stop bleeding by forming primary hemostatic plugs and by localizing thrombin for secondary hemostatic processes.[8]

Freeze-dried platelets have been studied in a number of animal models. In rabbits, the infusion of 40 to 50 $\times$ $10^9$ platelets shortened the ear bleeding time in thrombocytopenic rabbits from >900 seconds to a mean of 234 seconds (rehydrated platelets) and 177 seconds for fresh platelets.[9] Also noted was that the freeze-dried platelet recovery at 1 hour was 58%, compared with 79% for fresh human platelets. In splenectomized canines on cardiopulmonary bypass with prolonged bleeding times, infusion of freeze-dried platelets showed consistent and persistent lowering of bleeding times (from 7 minutes to 3 minutes 10 seconds, $p = 0.01$) compared with control.[10] In swine using a normovolemic dilutional coagulopathy model (blood exchange with hemoglobin-based oxygen carriers 40 mL/min), bleeding times were improved from >10 minutes down to 5 minutes to 7 minutes after freeze-dried platelet administration.[11] In another swine model of noncompressible hemorrhage, animals receiving freeze-dried platelets (compared with saline) were shown to have decreased total blood loss, improved control of hemorrhage (60% vs. 0%), and improved survival at 6 hours (80% vs. 20%). In this study, one of eight surviving pigs treated with freeze-dried platelets had evidence of thrombosis on necropsy.[12] In baboons, freeze-dried platelets exhibited modification of the platelet membrane that interfered with aggregation and thromboxane production, prevented increases in platelet P-selectin and glycoprotein inhibitor (GPI) Ib-IIIa, prevented decreases in GPIb after stimulation, and increased fenvalerate accumu-

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lation.[13] Such findings correlate with reduced survival in vivo of freeze-dried platelets.[14] Finally, in a dose safety study, 1.5× the endogenous platelet volume of freeze-dried platelets was infused into Cynomolgus monkeys (Huntingdon Life Sciences, Inc. Princeton, NJ). The monkeys showed no changes in clinical hemodynamic status, no elevations in inflammatory markers, and no evidence of disseminated intravascular coagulation. However, it is notable that on sacrifice, the spleens of the monkeys were found to be markedly enlarged.

The development of a safe and effective freeze-dried platelet product has been an elusive goal for the past half century. Freeze-dried platelets show some promise as hemostatic agents. Their short duration of action and circulation time may not present a barrier for acute indications such as trauma or dilutional coagulopathy. However, their partial functionality, possibly excess thrombogenicity, tendency to accumulate rapidly in the spleen, and antigenicity raise concerns about their safety.[15] Clinical studies are needed to show safety and efficacy in humans.

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